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CHEMICAL COMPOSITION AND BIOACTIVITY OF ESSENTIAL OIL FROM *PIPER UMBELLATUM* AGAINST GRAIN STORAGE INSECTS

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ABSTRACT

Insect pests are major constraints on crop production, the use of synthetic insecticides as a control against this pest has resulted in environmental concerns. The insecticidal activities of the essential oil of *Piper umbellatum* were investigated in the laboratory against bean weevil (*Callosbruchus maculatus*) and rice weevil (*Sitophilus oryzae*). The essential oil from *P. umbellatum* was extracted by steam distillation and graded into different concentrations, 1, 2, 2.5, 3, 4, 5, 7.5, 10 ml/l. The chemical component of the oil was analyzed by GC-MS. The toxicity of each dose was evaluated against bean weevil (*C. maculatus*) and rice weevil (*S. oryzae*) at different exposure time. The essential oil was toxic to both insects, toxicity of the essential oil was dose and time dependent ($P<0.05$). Essential oil of *P. umbellatum* induces higher toxicity in bean weevil than in rice weevil in all assay conducted. Analysis by GC-MS revealed the presence of 37 components in the essential oil, the major component are aromadendrene (13.74%), caryophyllene (10.44%), linalool (8.55%) and γ-bisabolene (8.06%). Based on present study results, it is suggested that the plant is suitable for possible use as insect pest control.

Keywords: Essential oil, insect pest, mortality, toxicity, fumigative, contact and component.

INTRODUCTION

In most developing countries in the world most especially in Africa, farm produce are damaged due to the invasion of insects during storage which as lead to huge economic loss to farmers (Owolabi *et al*., 2009). It is then important to find ways to ensure that farm produce are of high quality in terms of storage. The use of synthetic insecticides have been over the years used to combat the damage caused by these pest, and some of these chemicals have been found to be carcinogenic due to the toxic residue they leave on farm products (Owolabi *et al*., 2009).

Due to the enormous documented environmental risks that has been created by the use of synthetic pesticides in terms of toxicity in the environment, increase in the cost of usage as well as its effect on non-target organism (Jembere *et al*., 1995; Okonkwo and Okoye, 1996). There is a growing interest to make use of plant extracts which are biodegradable and from scientific findings have been found not to posses similar environmental risk like the synthetic chemicals (Lajide *et al*., 1998; Huang *et al*., 2000; Lee *et al*., 2001; Regnault-Roger *et al*., 2002 and Ngamo *et al*., 2007). Essential oils are plant extracts that posses mixture of terpenes and have been found useful in medicine, pharmaceutical, cosmetics and other areas due to their lower toxicity and persistence in the environment (Miyakado *et al*., 1997; Isman, 2000; Erler, 2005; Isikber *et al*., 2006).

The present study was carried out to determine the repellence, fumigative and contact toxicity activities of the essential oil of *P. umbellatum* in relation to exposure time at different concentrations against rice and bean weevil.

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MATERIALS AND METHODS

Plant material

P. umbellatum seeds were gotten from Orisumbare market of Osogbo, Osun State. The seed was identified by the Federal Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The seeds were air-dried and ground to a powder.

Insects culture

The founding insect culture of Bean weevil (*C. maculatus*) and Rice weevil (*S. oryzae*) was collected from infested beans and rice respectively. They were stored in a 5-liter plastic container and stored at a temperature of 24°C and 70% humidity.

Essential oil distillation
The ground powder of were subjected to hydrodistillation using a modified Clevenger-type apparatus for 6 h. Anhydrous sodium sulphate was used to remove water after extraction. Essential oils were stored in airtight containers in a refrigerator at 4°C.

GC-MS analysis
The essential oil was identified and quantified using Gas chromatography and mass spectrometry with an Agilent 6890N instrument equipped with a flame ionization detector and HP-5MS (30m × 0.25mm × 0.25µm) capillary column and Agilent Technologies 5973N mass spectrometer.

STATISTICAL ANALYSIS

The data were corrected using Abbott's formula (Abbot 1925) for the mortalities in the controls, and the data were subjected to probit analyses using SPSS (2001) for Windows to estimate LD_{50} and LD_{95} values of the essential oils against each stored-product insect species. Percentage mortality values for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program (SPSS 2001) for probit analysis.

Contact toxicity
The contact toxicity of the essential oil against bean weevil (*C. maculatus*) was evaluated on filter paper disc by treating a whatman No.1 filter paper with the essential oil diluted in 100% acetone. A micropipette was used to suck out 2, 4, 6, 8µl and 10µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 1, 2, 3, 4ml/L, and 5ml/L, respectively. They were each poured and allowed to flow regularly on a disc of filter paper placed in a petri dish. The solvent was allowed to dry after which 10 bean weevils were introduced into the petri dish and then closed. Percentage mortality of insects was observed every 10 minutes. Insects which did not respond to the gentle touch of a small probe were considered dead (Su, 1976). Each experiment was conducted in triplicate. Control experiment was done using only acetone. The same test was repeated for rice weevil (*S. oryzae*) but 5, 10, 15, 20µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 2.5, 5, 7.5ml/L and 10ml/L respectively to formulate insecticide.

Fumigative activity
Direct exposure of insects to vapors from essential oils and their chemical components was done with a small, sealed 1.5 L glass jar. A micropipette was used to suck out 2µl, 4µl, 6µl, 8µl and 10 µl this was diluted with 2ml of acetone to form concentrations of 1, 2, 3, 4ml/L, and 5ml/L. They were each poured and allowed to flow regularly on a disc of filter paper (whatman No. 1) placed upwardly in the top cover of the glass jar. After this application, 10 *C. maculatus* were introduced into the glass jar. The percentage mortality of insects was observed every 15 minutes. Each experiment was conducted in triplicate. Control experiment was done using only acetone. The test was repeated for rice weevil (*S. oryzae*) but 5, 10, 15, 20µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 2.5, 5, 7.5ml/L and 10ml/L, respectively to formulate insecticide.

Repellent activity
The repellent effects of the essential oil against beans weevils (*C. maculatus*) were evaluated using the area preference method. Tested areas consisting of Whatman No.1 filter paper cut in half 2, 4, 6, 8µl and 10µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 1, 2, 3, 4ml/L, and 5ml/L, respectively to formulate insecticide while for rice weevil, 5, 10, 15, 20µl of the essential oil and was diluted with 2ml of acetone to form concentrations of 2.5, 5, 7.5ml/L and 10ml/L, respectively to formulate insecticide. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. 10 adult insects of each species were released separately at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in incubator at 27 ± 2°C and 75 ± 5% relative humidity.

RESULTS AND DISCUSSION

All graded concentrations caused mortality in *C. maculates* and *S. oryzae* in the contact treatment (Tables 1 and 2) the percentage mean mortality is dose and time dependent. The concentration of 5ml/l recorded 100% mortality of *C. maculates* after 36 Hrs of exposure, on the other hand, subjecting *S. oryzae* to the same concentration resulted in 100% mortality after 72 Hrs of exposure. The essential oil of *P. umbellatum* was more toxic against *C. maculates* than in *S. oryzae* in the contact treatment as revealed by the LD_{50} and LD_{95}. The lethal doses of *P. umbellatum* against *C. maculatus* after 24 Hrs of exposure are 3.76ml/l (at LD_{50}) and 5.63ml/l (at LD_{95}) while those of *S. oryzae* are 7.57ml/l (at LD_{50}) and 10.88 (at LD_{95}) this was significant at $P < 0.05$.

The percentage mortality of *C. maculatus* and *S. oryzae* due to the fumigative activity of the essential oil of *P. umbellatum* is recorded in Tables 3 and 4 respectively. The result revealed that the mortality of the *C. maculatus* and *S. oryzae* were dose and time dependent. The mortality of both insects increased with concentration and time, a dose of 5ml/l of the essential oil against *C. maculatus* in the fumigative treatment (Table 3) recorded 100% mortality of *C. maculatus* on the 4th day of exposure to the essential oil while for *S. oryzae* using the same dose, 100% mortality was observed on the 6th day. These results showed that the essential oil of *P.*

Table 1. The percentage mortality of contact activity of the *P. umbellatum* essential oil against *C. maculatus).*

Exposure Time (hrs)	Conc (mL/L)					
	1	2	3	4	5	Control
12	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	37.5±5.8^{b}	75.0±10.0^{d}	0.0±0.0^{a}
24	0.0±0.0^{a}	12.5±5.8^{b}	12.5±5.8^{b}	62.5±5.8^{c}	87.5±5.8^{d}	0.0±0.0^{a}
36	12.5±5.8^{b}	50.0±5.8^{cd}	62.5±5.8^{d}	87.5±10.0^{e}	100±0.0^{f}	0.0±0.0^{a}
48	50±10.0^{c}	87.5±5.8^{d}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	0.0±0.0^{a}
60	75±5.8^{c}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	12.5±5.8^{a}
72	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	12.5±5.8^{a}

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P <0.05.

Table 2. The percentage mortality of contact activity of the *P. umbellatum* essential oil against *S.oryzae*.

Exposure Time (hrs)	Conc (mL/L)				
	2.5	5	7.5	10	Control
12	0.0±0.0^{a}	0.0±0.0^{a}	12.5±5.8^{b}	25.0±5.8^{c}	0.0±0.0^{a}
24	0.0±0.0^{a}	12.5±5.8^{b}	50.0±5.8^{c}	87.5±5.8^{d}	0.0±0.0^{a}
36	12.5±5.8^{b}	25.0±5.8^{c}	62.5±10.0^{d}	100±0.0^{e}	0.0±0.0^{a}
48	37.5±5.8^{c}	62.5±10.0^{d}	100±0.0^{e}	100±0.0^{e}	0.0±0.0^{a}
60	62.5±5.8^{c}	87.5±5.8^{d}	100±0.0^{e}	100±0.0^{e}	0.0±0.0^{a}
72	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	0.0±0.0^{a}

Table 3. The percentage mortality of fumigant activity of the *P. umbellatum* essential oil against *C. maculatus*).

Exposure Time(Days)	Conc (mL/L)					
	1	2	3	4	5	Control
1	0.0±0.0^{a}	0.0±0.0^{a}	12.5±5.8^{b}	25.0±5.8^{c}	37.5±5.8^{d}	0.0±0.0^{a}
2	0.0±0.0^{a}	25.0±5.8^{b}	50.0±5.8^{c}	62.5±5.8^{d}	62.5±5.8^{d}	0.0±0.0^{a}
3	25.0±5.8^{b}	37.5±5.8^{c}	75.0±10.0^{d}	87.5±5.8^{de}	87.5±5.8^{de}	0.0±0.0^{a}
4	50.0±5.8^{c}	62.5±10.0^{cd}	87.5±5.8^{d}	100±0.0^{e}	100±0.0^{e}	6.3±5.8^{a}
5	100±0.0^{d}	100±0.0^{d}	100±0.0^{d}	100±0.0^{d}	100±0.0^{d}	12.5±5.8^{a}

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P <0.05.

Table 4. The percentage mortality of fumigant activity of the *Piper umbellatum essential*oil against *S. oryzae*.

Exposure Time(Days)	Conc (ml/l)				
	2.5	5	7.5	10	Control
1	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	25.0±5.8^{b}	0.0±0.0^{a}
2	0.0±0.0^{a}	12.5±5.8^{b}	25.0±5.8^{c}	50.0±5.8^{d}	0.0±0.0^{a}
3	0.0±0.0^{a}	37.5±5.8^{b}	62.5±5.8^{c}	62.5±5.8^{c}	0.0±0.0^{a}
4	12.5±5.8^{b}	50.0±5.8^{c}	87.5±5.8^{d}	100±0.0^{e}	0.0±0.0^{a}
5	62.5±5.8^{c}	75.0±10.0^{d}	100±0.0^{e}	100±0.0^{e}	0.0±0.0^{a}
6	100±0.0^{d}	100±0.0^{d}	100±0.0^{d}	100±0.0^{d}	6.7±0.0^{a}

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P < 0.05.

umbellatum was more toxic against *C. maculates* as revealed by the LD_{50} and LD_{95}. The lethal doses of *P. umbellatum* against *C. maculates* after 24 hrs of exposure to the essential oil were 3.66ml/l (at LD_{50}) and 6.99ml/l (at LD_{95}) while those of *S. oryzae* were 9.88ml/l (at LD_{50}) and 15.04 (at LD_{95}). The study is in agreement with what was reported by Papachristes and Stamopoulus 2002.

Table 5. The percentage repellent activity of the *P. umbellatum* essential *oil* against *C. maculatus*).

Exposure Time(Mins)	Conc (ml/l)				
	1	2	3	4	5
15	0.0±0.0^{a}	0.0±0.0^{a}	25.0±5.8^{b}	50.0±5.8^{c}	62.5±5.8^{d}
30	12.5±5.8^{a}	25.0±5.8^{b}	62.5±5.8^{c}	87.5±5.8^{d}	100±0.0^{e}
45	50.0±10.0^{c}	62.5±5.8^{c}	87.5±5.8^{d}	100±0.0^{e}	100±0.0^{e}
60	81.5±5.8^{d}	100±0.0^{e}	100±0.0^{e}	100.0±0.0^{e}	100±0.0^{e}
72	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}	100±0.0^{e}

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

Table 6. The percentage repellent activity of the *P. umbellatum* oil against rice weevils *S. oryzae*).

Exposure Time(Mins)	Conc (ml/l)			
	2.5	5	7.5	10
30	0.0±0.0^{a}	16.2±5.8^{b}	46.9±5.8^{c}	75±5.8^{d}
60	16.2±5.8^{b}	46.2±5.8^{c}	66.7±5.8^{d}	94.7±5.8^{e}
90	44.4±5.8^{c}	62.7±5.8^{cd}	82.4±5.8^{de}	100±0.0^{ef}
120	75.0±6.0^{c}	82.4±5.8^{cd}	82.4±5.8^{cd}	100±0.0^{e}
150	82.4±5.8^{d}	94.7±5.8^{d}	100±0.0^{e}	100±0.0^{e}

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

Tables 5 and 6 shows the repellence activity of the essential oil of *P. umbellatum* against *C. maculates* and *S. oryzae* with the repellent property being also dose and time dependent. A dose of 5ml/l against *C. maculates* revealed 100% mortality repellence after 30 minutes (Table 5) but against *S. oryzae* 16.5% repellence activity was observed after 30 minutes (Table 6). This shows that the essential oil of *P. umbellatum* evoked higher repellency in *C. maculatus* than in *S. oryzae.* The results of the contact, fumigative and repellent test revealed that the effect of the essential oil as compared with the control was significant at (P<0.05). From Table 7 thirty seven components were detected from the GC-MS analysis of *Piper umbellatum* essential oil. The major component are aromadendrene (13.74%), caryophyllene (10.44%), linalool (8.55%) and γ-bisabolene (8.06%). Martin et al, 1998 showed that the major components from the seed of *P. umbellatum* are β-pinene, α-pinene, caryophellene and linalool same was reported by Tchoumbougnang *et al*., 2009.

In insect pest management in agriculture, the direct contact of the insects with the insecticide plays an important role in achieving a high mortality, but residue left on food is a huge concern to man. The biodegradable nature of essential oils and its volatility encourages its use as an insecticide, but several questions comes to mind for example, how long will it take for the essential oil to show its first sign of toxicity on the insects?, when will the essential oil exhibit its highest toxicity?, and how long will the effectiveness of the toxicity lapse. This question forms the backbone of this research.

In this study, the contact bioassay have shown that essential oil of *P. umbellatum* started to exhibit toxicity (12.5% mortality) against *C. maculatus* after 36 Hrs of exposure when a dose of 1ml/l was used, with the same dose, 100% mortality was recorded after 72 Hrs of exposure in *C. maculates* . In the case of *S. oryzae,* initial toxicity of the essential oil was observed after 36 Hrs (Table 2) when the insect was exposed to a dose of 2.5ml/l in the contact treatment. The highest toxicity (100%) in *S. oryzae* was observed after 72 hrs of exposure to the same dose of essential oil. The results showed that the effectiveness of the essential oil was not lost days after application and the effectiveness increase with time.

In the control of insect pest in bags containing seeds for storage, it is important that insects which are not in direct contact with the formulated insecticides are affected by the insecticides so as to prevent damage during storage. This can only be possible if the insecticide releases fumes which will cause toxicity on the insects where ever it's hidden. The bioassay on the fumigative effect of essential oil of *P. umbellatum* (Tables 3 and 4) revealed that the essential oil showed toxicity in all treatment. *C. maculatus* showed first sign of toxicity (25%) in 3 days with a dose of 1ml/l, maximum toxicity (100%) was observed on the 5th day of exposure. For *S. oryzae* lowest toxicity of 12.5% was observed on the 4th day on

Table 7. The chemical component of *Piper umbellatum* essential oil.

Compound Present	Retention Time(Min)	% Composition
Linalool	3.322	8.55
Bicyclo[3.1.0]hex-2-ene, 4,4,6,6-tetramethyl	3.476	0.34
Alpha.-cubebene	7.115	0.44
Copaene	7.630	2.15
Cyclohexane, 1-ethenyl-1-methyl-2, 4 bis (1methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]	8.134	4.53
1H-Cycloprop[e]azulene, decahydro- 1,1,7-trimethyl-4-methylene-, [1Ar (1a.alpha.,4a.alpha.,7.alpha.,7a. beta.,7b.alpha.)]	8.254	1.38
Caryophyllene	8.660	10.44
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR 1a.alpha.,4a.beta.,7.alpha.,7a.b eta.,7b.alpha.)]-	8.895	1.43
4,7-Methanoazulene, 1,2,3,4,5,6,7, 8-octahydro-1,4,9,9-tetramethyl-[1S-(1.alpha.,4.alpha.,7.alpha.)]-	9.101	4.75
Aromadendrene	9.444	13.74
Hydroxylamine, O-decyl- Dodecane, 1-fluoro- Hexadecane, 1-chloro-	9.644	0.91
1,6-Cyclodecadiene, 1-methyl-5-met hylene-8-(1-methylethyl)-, [s-(E,E)]-	9.890	6.56
Naphthalene, decahydro-4a-methyl-1 -methylene-7-(1-methylethenyl)-, [4aR(4a.alpha.,7.alpha.,8a.beta.)]	9.959	2.25
1H-Cyclopropa[a]naphthalene, decah ydro-1,1,3a-trimethyl-7-methylene- [1aS(1a.alpha.,3a.alpha.,7a.bet a.,7b.alpha.)]-	10.068	3.81
Gamma-Bisabolene	10.342	8.06
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	10.503	0.82
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S(R*,S*)]-	10.651	4.58
Cis-.alpha.-bisabolene	10.880	0.68
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	10.789	0.35
Gamma.-elemene	11.149	0.71
6,7-Dimethox y-2-oxo-2H-chromen-4-arbaldehyde	11.349	0.43
Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	11.481	0.61
Nerolidol	11.607	3.47
Caryophyllene oxide	11.876	1.63
(-)-Spathulenol	11.962	0.50
Guaiol	12.145	1.90
12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	12.345	0.74
1-Octadecanesulphonyl chloride	12.637	0.48
Tricyclo[4.1.0.0(2,4)]heptane, 3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)-, (1.alpha.,2.beta.,4.beta. ,5.alpha.,6.alpha.)-	12.849	0.55
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1R-(1.alpha.,3a.beta.,4.alpha.7.beta)]	13.072	0.56
Alpha.-cadinol	13.158	0.91
n-Hexadecanoic Acid	17.501	3.29

exposure to a dose of 2.5ml/l, maximum mortality was observed after 6 days of exposure to the treatment.

The mortality observed in the contact treatment could be as a result of volatile constituent entering the cuticle of the insects. The higher susceptible of the *C. maculatus* to the essential oil could be as a result of its softer cuticle that allows easier penetration of the essential oil, while the fumigative activity could be as a result of the volatile fumes of the essential oil entering the spiracles and trachea of the insects. The mortality of insect could be as a result of the nerve impulse by inhibition of

acetylcholine. This impulse lead to paralysis and the death of the insects (Keane and Ryan, 1999). The repellence activity of the essential oil could be as a result of the pungent odour which drives away the insects away from which the smell is strongest. The repellence activity was also time and dose dependent. This is the same as reported by Asawalam *et al*. (2006). The result indicated a higher repellence in bean weevil than in rice weevil.

The activities of the essential oil could be as result of the volatile constituent of the essential oil. Tapondjou *et al*. (2002) reported that the toxicity of an essential oil depends on the biological active plant components present in the species and on the treated insects. These study shows that *S. oryzae* is tolerant against the essential oil of *P. umbellatum* compared to *C. maculatus.* This result is in agreement with (Ayvaz *et al*., 2009) who observed an increase in mortality of *C. maculates* adults with increase concentrations of essential oil and exposure time.

CONCLUSION

In conclusion our results suggested that potential future application of these essential oil or their active components for the control of insect pest may exploit more than one mode of action. However, future experiments should focus on fractionating the different components and then testing the different components on more target insects to determine the component(s) responsible for the insecticidal activities.

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